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*ON THE SIGNIFICANCE OF AVERAGE MOLECULAR WEIGHTS FROM SEDIMENTATION EQUILIBRIUM EXPERIMENTS**

BY E. T. ADAMS, JR.†

CHEMISTRY DEPARTMENT, UNIVERSITY OF WISCONSIN, MADISON

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It is proposed here to call attention to certain precautions which require consideration in interpreting average molecular weights from sedimentation equilibrium experiments (Parts 1 and 2), and to demonstrate the existence of a new type average which is derivable from equations which could lead to the determination of the number average molecular weight (Part 3). The discussion is restricted to ideal, incompressible solutions with a polydisperse macromolecular component. No review of the theory basic to sedimentation equilibrium is presented, since this information is readily available.[1-3]

1. *Average Molecular Weights Over the Cell for Nonassociating Solutes.*—Here is considered elaboration of statements of Lansing and Kraemer[4] regarding the existence of *two different* weight average (or any other average) molecular weights over the cell, one of which represents the average molecular weight of the original solution, while the other does not.

The usual $M_{w\ cell\ mass}$ describes an average molecular weight over the *mass* of solute in the solution column in the ultracentrifuge cell, thus

$$M_{w\ cell} = \frac{\int_a^b M_{wr} c_r d(r^2)}{\int_a^b c_r d(r^2)} = \frac{\int_a^b M_{wr} dm}{\int_a^b dm}$$

$$= \frac{(c_b - c_a)2RT}{c_0(1 - \bar{v}\rho)\omega^2(b^2 - a^2)} \qquad (1)$$

in which $\bar{v}$ = partial specific volume; ρ = density of the solution; M_{wr} = weight average molecular weight at any radial position, r, in the cell; c_r = concentration at position r; $dm = \theta hcd(r^2)/2 = c\,dV$; b = radial distance to cell bottom; a = radial position of the meniscus; and ω = angular velocity.

In arriving at this equation it was assumed that all the solute partial specific volumes are equal and the quantity $(1 - \bar{V}\rho)$ is constant.[5] For ideal, nonassociating solutes, it is readily shown that $M_{w\ cell}$ represents the original weight average molecular weight of the sample. Required to this end are the definitions of the

weight average molecular weight, and a statement of the conservation of mass for each component, i. Thus,

$$M_{w\,cell} = \sum M_i \int_a^b c_{i,r}d(r^2)/\int_a^b c_{i,r}d(r^2) = \frac{\sum M_i c_{i,0}(b^2 - a^2)}{\sum c_{i,0}(b^2 - a^2)}. \tag{1a}$$

In this equation the symbol $c_{i,0}$ represents the initial concentration of the solute species i.

If one were to evaluate equation (1) numerically by using equal intervals in r^2,

$$M_{w\,cell} \cong \frac{\Delta(r^2)\sum_{i=1}^{n} c_{ri}M_{wri}}{\Delta(r^2)\sum_{i=1}^{n} c_{ri}} = \frac{\sum_{i=1}^{n} c_{ri}M_{wri}}{\sum_{i=1}^{n} c_{ri}}. \tag{1b}$$

As the intervals in $\Delta(r^2)$ become infinitely small, equation (1b) becomes identical with equation (1), and it is seen that $M_{w\,cell}$ represents a weighted average, where c_r is the weighting factor.

On the other hand, one can also define a weight average molecular weight over the volume of the solution column. Thus,

$$M_{w\,cell\,vol} = \frac{\int_a^b M_{wr}d(r^2)}{\int_a^b d(r^2)} = (1/A)\ln(c_b/c_a)/(b^2 - a^2). \tag{2}$$

By numerical evaluation of equation (2) in equal intervals of (r^2), one finds,

$$M_{w\,cell\,vol} \cong \frac{\Delta(r^2)}{n\Delta(r^2)}\sum_{i=1}^{n} M_{wri} = \sum_{i=1}^{n} M_{wri}/n \tag{2a}$$

Thus, $M_{w\,cell\,vol}$ may be thought of as a numerical average of M_{wr} in the solution column.

However, there is a difference between $M_{w\,cell\,mass}$ and $M_{w\,cell\,vol}$. For each solute species the following equation holds:

$$\frac{c_{bi} - c_{ai}}{c_{oi}} = \ln(c_{bi}/c_{ai}) = AM_i(b^2 - a^2), \tag{3}$$

where $A = (1 - \bar{v}\rho)\omega^2/2RT$. For q solutes, we have

$$\sum_{i=1}^{q} c_{bi} - \sum_{i=1}^{q} c_{ai} = c_b - c_a = \sum_{i=1}^{q} c_{oi}\ln(c_{bi}/c_{ai}). \tag{3a}$$

It then follows that

$$\ln(c_b/c_a) \neq (c_b - c_a)/c_0. \tag{4}$$

except for monodisperse ideal solutions. So, it is apparent from the inequality (4), as well as from a comparison of equations (1) and (2), that the two average molecular weights are different. The quantity $M_{w\,cell}$, equation (1), is larger than the quantity $M_{w\,cell\,vol}$, equation (2), since weighted averages are larger than numerical averages. The quantity $M_{w\,cell}$ is a parameter of the weight and number distribution curves; for further details one may refer to the Fujita monograph.[3] The statement by Stille[6] that the weight average molecular weight of polydisperse

solutes is given by equation (2), or the presumed use of equation (2) by Hexner and co-workers[7] to compute M_w for a polydisperse solute (polystyrene) must be carefully evaluated.

Similar distinctions hold for other average molecular weights. Thus, remembering that $M_z = [d(cM_w)]/dc$,

$$M_{z\,cell} = \frac{(cM_w)_b - (cM_w)_a}{c_b - c_a} = \left(\frac{1}{2A}\right)\left\{\frac{\left(\frac{1}{r}\frac{dc}{dr}\right)_b - \left(\frac{1}{r}\frac{dc}{dr}\right)_a}{c_b - c_a}\right\}. \quad (5)$$

This average was introduced by Lansing and Kraemer.[4]

For the corresponding volume-based average we have,

$$M_{z\,cell\ vol} = \int_a^b M_{zr}d(r^2)/\int_a^b d(r^2) = \frac{\ln\left(\frac{1}{r}\frac{dc}{dr}\right)_b/\left(\frac{1}{r}\frac{dc}{dr}\right)_a}{A(b^2 - a^2)}. \quad (6)$$

The usual quantity $M_{z\,cell}$, equation (5), represents a weighted average of M_{zr} in the solution column, with the quantity $z_r = \sum_{i=1}^{q} c_{i,r}\cdot M_i$ being the weighting factor. On the other hand, equation (6) shows that $M_{z\,cell\ vol}$ represents a numerical average of M_{zr} in the solution column.

2. *Average Molecular Weights in Associating Systems.*—Many proteins undergo reactions of the type $nP \rightleftarrows P_n$ to form polymeric species which exist in rapid dynamic equilibrium with the monomeric species. In a few instances, cases of the combined sedimentation and chemical equilibrium have been observed.[8-12] In the attempt to describe the chemical equilibria one makes use of average molecular weights. The quantity which is usually sought is the weight average molecular weight over the cell, $M_{w\,cell}$, equation (1). However, for ideal associating solutes and as defined above, it can be shown that $M_{w\,cell}$ does not represent the original weight average molecular weight of the sample. We note first that if all partial specific volumes are equal, an assumption one is forced to make in associating systems (since one generally measures partial specific volumes in a solution containing an equilibrium mixture of all associating species), the equation for the total concentration at any radial position for a monomer—n-mer equilibrium becomes[13, 14]

$$c_r = c_{1a}\exp\{AM_1(r^2 - a^2)\} + K_{1n}c_{1a}^{\,n}\exp\{nAM_1(r^2 - a^2)\}, \quad (7)$$

where

$$c_r = c_{1r} + c_{nr} = c_{1r} + K_{1n}c_{1r}^{\,n}$$

$$K_{1n} = c_1^{\,n}/c_1 \text{ for } nP \rightleftarrows P_n$$

$$c_{r_0} \equiv c_0 = c_{1a}\exp\{AM_1(r_0^2 - a^2)\} + K_{1n}c_{1a}^{\,n}\exp\{nAM_1(r_0^2 - a^2)\}. \quad (7a)$$

Here, the subscript 1 refers to the monomer and n to the n-mer. It should be noted that we have used the meniscus as the lower limit of integration in writing down equation (7); Tiselius[13] and Svedberg and Pedersen[14] here preferred to use b and r as their limits for the required integration.

Now the conservation of mass also gives an equation for c_0. Thus,

$$\int_a^b c_r d(r^2) = \int_a^b (c_{1r} + K_{1n}c_{1r}^n)d(r^2) = (c_{1a}/AM_1)[\exp\{AM_1(b^2 - a^2)\} - 1] + (K_{1n}c_{1a}^n/nAM_1)\ [\exp\{nAM_1(b^2 - a^2)\} - 1]. \quad (8)$$

It should be noted that

$$\left(\frac{c_{1a}[\exp\{AM_1(b^2 - a^2)\} - 1]}{AM_1(b^2 - a^2)}\right)^n \neq \frac{c_{1a}^n[\exp\{nAM_1(b^2 - a^2)\} - 1]}{nAM_1(b^2 - a^2)}. \quad (9)$$

The inequality (9) and a term-by-term comparison of equations (7*a*) and (8) tell us that

$$c_{01} \equiv c_{1a} \exp AM_1(r_0^2 - a^2) \neq \frac{c_{1a}[\exp\{AM_1(b^2 - a^2)\} - 1]}{AM_1(b^2 - a^2)} \quad (10)$$

and

$$c_{01}^n \equiv c_{1a}^n \exp\{nAM_1(r_0^2 - a^2)\} \neq \frac{c_{1a}^n[\exp\{nAM_1(b^2 - a^2)\} - 1]}{nAM_1(b^2 - a^2)}. \quad (11)$$

For $M_{w\ cell}$ to be the weight average molecular weight at the original concentration, $M_{w\ cell}$ would have to obey the following relation:

$$M_w \text{ (at } c = c_0) = M_1(c_{0,1} + nK_{1n}c_{0,1}^n)/c_0, \quad (12)$$

which is not the case. Actually,

$$M_{w\ cell} \int_a^b c_r d(r^2) = \int_a^b M_{wr} c_r d(r^2) \quad (13)$$

$$c_0(b^2 - a^2)M_{w\ cell} = M_1 \int_a^b (c_{1r} + K_{1n}c_{1r}^n)d(r^2) \quad (14)$$

$$M_{w\ cell} = \frac{M_1}{c_0}\left\{\frac{c_{1a}[\exp\{AM_1(b^2 - a^2)\} - 1]}{AM_1(b^2 - a^2)} + \frac{nK_{1n}c_{1a}^n[\exp\{nAM_1(b^2 - a^2)\} - 1]}{nAM_1(b^2 - a^2)}\right\}. \quad (15)$$

A comparison of equation (15) with the inequalities (10) and (11) shows that equation (15) cannot satisfy equation (12). Thus, although one can apply the conventional formula for computing $M_{w\ cell}$ in associating systems, the quantity so obtained does not have the same significance as it does in the nonassociating case. Furthermore, it means one must use the less precise M_{wr} to evaluate equilibrium constants and to obtain information for associating systems at sedimentation equilibrium.

3. *Evaluation of M_n and Some New-Type Average Molecular Weights.*—Fujita[15] has suggested that the number average molecular weight, M_n, may be evaluated from a set of sedimentation equilibrium experiments at various speeds and/or solution column thicknesses; his equations for the evaluation of M_n are based on schlieren optics. It will be demonstrated here how, at least in principle, the numerical value of M_n can be obtained by using Rayleigh optics or any other optical system that gives data directly proportional to concentration. We start with Fujita's[3] equation (5.170).

$$c(\zeta) = c_0 \sum_{i=1}^{q} \frac{\lambda M_i f_i^0 \exp(-\lambda M_i \zeta)}{1 - \exp(-\lambda M_i)} \tag{16}$$

where

$$\zeta = (b^2 - r^2)/(b^2 - a^2), \qquad f_i^0 = c_i/c_0$$

and

$$\lambda = (1 - \bar{v}\rho)\omega^2(b^2 - a^2)/2RT.$$

Then,

$$c(\zeta = 1/2) = c_0 \sum_{i=1}^{q} (\lambda M_i/2) f_i^0 \sinh(\lambda M_i/2).$$

If we let $x_i = \lambda M_i/2$ and $d\lambda = 2dx_i/M_i$, we have

$$\int_0^\infty \frac{c(\zeta = 1/2)}{c_0}\, d\lambda = \int_{x_i=0}^{x_i=\infty} \sum_{i=1}^{q} \frac{2x_i dx_i f_i^0}{\sinh x_i M_i}$$

$$= \sum_{i=1}^{q} \frac{f_i^0}{M_i} \int_{x_i=0}^{x_i=\infty} \frac{2x_i dx_i}{\sinh x_i} = \pi^2/2M_n. \tag{17}$$

In addition it should be noted that one can use the meniscus concentration, $c(\zeta = 1)$, as well to evaluate M_n. Thus,

$$\int_0^\infty \frac{c(\zeta = 1)}{c_0}\, d\lambda = \int_{\lambda=0}^{\lambda=\infty} \sum_{i=1}^{q} \frac{f_i^0 z_i}{\exp z_i - 1}\, d\lambda$$

$$= \sum_{i=1}^{q} \frac{f_i^0}{M_i} \int_{z_i=0}^{z_i=\infty} \frac{z_i dz_i}{\exp z_i - 1} = \pi^2/6M_n. \tag{18}$$

Although equation (17) or (18) will give M_n, there are practical difficulties which prevent their successful application. We note that λ must approach infinity. The concomitant increase in speed causes such an increase in concentration at the centrifugal end of the usual 12-mm cell that the fringes will be defocused in this region. Thus, it will be difficult to apply the conservation of mass to determine the concentrations at the meniscus radial position, where $\zeta = 1$, or at the radial position, where $\zeta = 1/2$. This difficulty can perhaps be overcome, since

$$\int_0^\infty \left[\frac{c(\zeta = 1/2) - c(\zeta = 1)}{c_0}\right] d\lambda = \pi^2/2M_n - \pi^2/6M_n = \pi^2/3M_n. \tag{19}$$

In fringe notation equation (19) becomes

$$\int_0^\infty \left[\frac{J(\zeta = 1/2) - J(\zeta = 1)}{J_0}\right] d\lambda = \pi^2/3M_n. \tag{19a}$$

Here, $J = h(n - n_0)/\Lambda$ = the number of interference fringes; h = cell thickness; Λ = wavelength of light; $n - n_0 = (\partial n/\partial c)c$ = excess refractive index of solution over that of the solvent; and $(\partial n/\partial c)$ = refractive increment. An additional virtue of equation (19) or (19a) is that it involves the difference in concentration or fringes between the meniscus ($\zeta = 1$) and the radial position where $\zeta = 1/2$; this concen-

tration difference is directly measurable from the photographic plate and does not require knowledge of the absolute values of the concentration or fringes at the two positions. Since one must evaluate the integral graphically in order to obtain M_n, it is of interest to note that the plot of $(1/J_0)\cdot(J(\zeta = 1/2) - J(\zeta = 1))$, or the corresponding concentration ratio, against λ begins and ends with the value zero for the ordinate and goes through one maximum. At the maximum the following relation obtains

$$\left[\frac{J(\zeta = 1/2) - J(\zeta = 1)}{J_0}\right]_{\max} = \sum_{i=1}^{q} \frac{f_i^0(\lambda^* M_i/2)^2 \cosh(\lambda^* M_i/2)}{\sinh^2(\lambda^* M_i/2)} - \sum_{i=1}^{q} \frac{f_i^0(\lambda^* M_i)^2 \exp(\lambda^* M_i)}{[\exp(\lambda^* M_i) - 1]^2} \quad (20)$$

in which λ^* = the value of λ at the maximum value of $\dfrac{J(\zeta = 1/2) - J(\zeta = 1)}{J_0}$.

If one takes the quantity $\dfrac{J(\zeta = 1/2) - J(\zeta = 1)}{J_0}$ and integrates it over λ^2, one obtains a new-type average molecular weight, M_{-1}. Thus,

$$\int_0^\infty \left[\frac{J(\zeta = 1/2) - J(\zeta = 1)}{J_0}\right] d(\lambda^2) = \sum_{i=1}^{q} \frac{f_i^0}{M_i^2}.$$

$$\left[\int_{x_i=0}^{x_i=\infty} \frac{8x_i dx_i}{\sinh x_i} - \int_{z_i=0}^{z_i=\infty} \frac{2z_i dz_i}{\exp z_i - 1}\right] = \frac{33.66 - 4.81}{M_{-1}} = \frac{28.85}{M_{-1}}, \quad (21)$$

where $M_{-1} = \sum_q c_i / \sum_q (c_i/M_i^2) = 1/\sum_q (f_i/M_i^2)$.

One can also obtain the quantity M_{-1} from schlieren optics; thus,

$$\int_0^\infty q(\lambda) d(\lambda^3) = 3\pi^4/M_{-1}$$

$$q(\lambda) = (-1/\lambda c_0)\left[\frac{dc(\zeta = 1/2)}{d\zeta}\right].$$

(This is equation 5.185 in the Fujita monograph.[3])

In principle one could generate as many of these average molecular weights as desired; however, the precision of the experiment will impose practical limits. For polydisperse solutes M_{-1} and M_{-2} are parameters that could be used to characterize the heterogeneity of the sample; they are related to moments of the distribution of molecular weights on a weight basis. The kth moment (ν_k) of the distribution of molecular weights on a weight basis $f(M)$ is

$$\nu_k = \int_0^\infty M^k f(M) dM.$$

Thus, for $k = -2$, $\nu_{-2} = 1/M_{-1}$.

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† Present address: Hercules Research Center, Wilmington, Delaware 19899.

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$$\bar{M}_1 = \frac{(c_b - c_a).2\,RT}{c_s\,(1 - \bar{v}_w\rho)\,\omega^2\,(b^2 - a^2)}.$$

Here, $\bar{v}_w$ = weight average partial specific volume

$$M_1 = \frac{\sum_q^q c_i M_i \phi_i\,(1 - \bar{v}_i\rho)}{\sum_q^q c_i\,\phi_i\,(1 - \bar{v}_i\rho)}$$

$\phi_i = \partial n/\partial c_i$ = refractive index increment for each solute species i

$\bar{v}_i$ = partial specific volume of each solute species

$(1 - \bar{v}_w\rho) = \sum c_i(1 - \bar{v}_i\rho)/c$

ρ = density of the solution.

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*TRANSIENT EPR AND ABSORBANCE CHANGES IN PHOTOSYNTHETIC BACTERIA**

BY R. H. RUBY, I. D. KUNTZ, JR.,† AND M. CALVIN

LAWRENCE RADIATION LABORATORY, DEPARTMENT OF PHYSICS, AND DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CALIFORNIA, BERKELEY

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Light-induced electron paramagnetic resonance (EPR) signals have been observed in photosynthetic systems for many years. These observations have been discussed in several recent review papers.[1, 2] A positive identification of the signal with a definite molecular species has proved difficult on the basis of EPR properties alone. Thus, correlations of these properties with other physical measurements